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Stimulation by oxygen radicals of prostaglandin production by rat renal glomeruli

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Stimulation by oxygen radicals of prostaglandin production by rat renal glomeruli. Polymorphonuclear leukocytes secreting oxygen radicals are found in the glomerular capillaries at an early stage of experimental acute glomerulonephritis. The aim of this work was to study the effects of these radicals on prostaglandin (PG) production by the glomeruli. Glomeruli were isolated from rat renal cortex and incubated in the presence of a biochemical system capable of generating oxygen radicals (addition to 100 μM xanthine of increasing concentrations of xanthine-oxidase). Synthesis of PGE_2, $PGF_{2\alpha}$, 6 keto $PGF_{1\alpha}$, and TXB_2 estimated using specific radioimmunoassays was twofold greater in the presence of oxygen radicals. This effect was inhibited by catalase, slightly stimulated by superoxide dismutase, and unaffected by hydroxyl radical scavengers, thus suggesting that hydrogen peroxide was the by-product responsible. This was confirmed by the stimulatory effect of hydrogen peroxide itself (1 to 100 μM) on PG synthesis. The effect of mepacrine, an inhibitor of phospholipase activity, on PG production was more marked in the presence of hydrogen peroxide and the stimulation of PG synthesis by hydrogen peroxide or oxygen radicals was progressively inhibited in the presence of arachidonic acid. Moreover, oxygen radicals stimulated the release of ^{14}C-arachidonic acid previously incorporated in isolated glomeruli. This demonstrates that the increase in PG synthesis in response to oxygen radicals is due to activation of glomerular phospholipase by these radicals. This effect that is likely to occur at an early stage of experimental glomerulonephritis could play a role in the mechanism of the inflammatory process.

Stimulation par les radicaux oxygène de la production de prostaglandines par les glomérules de rein de rat. Des polynucléaires sécrétant des radicaux oxygène libres s'accumulent dans les capillaires glomérulaires à une phase initiale des glomérulonéphrites aigues expérimentales. Le but de ce travail fut d'étudier les effets de ces radicaux sur la production de prostaglandines (PG) par les glomérules. Les glomérules ont été isolés à partir de cortex rénal de rat et incubés en présence d'un système biochimique capable de donner naissance à des radicaux oxygène libres (mélange de 100 μM de xanthine et de concentrations croissantes de xanthine-oxydase). La synthèse de PGE_2, $PGF_{2\alpha}$, 6 ceto $PGF_{1\alpha}$, et TXB_2 mesurée par dosage radioimmunologique spécifique est deux fois plus grande en présence de radicaux oxygène libres. Cet effet est inhibé par la catalase, légèrement stimulé par la superoxyde dismutase, et reste inchangé en présence de capteurs de radicaux hydroxyl, ce qui suggère que le peroxyde d'hydrogène est le métabolite responsable. Cela fut confirmé par l'action stimulatrice du peroxyde d'hydrogène lui-même (1 à 100 μM) sur la synthèse de PG. L'effet de la mépacrine, un inhibiteur de l'activité phospholipasique, sur la production de PG est plus marqué en présence de peroxyde d'hydrogène et la stimulation de la synthèse de PG par le peroxyde d'hydrogène ou les radicaux oxygène libres est progressivement inhibée en présence d'acide arachidonique. De plus, les radicaux oxygène libres stimulent la libération d'acide arachidonique ^{14}C préalablement incorporé dans les glomérules isolés. Cela démontre que l'accroissement de la synthèse de PG en réponse aux radicaux oxygène libres est dû à l'activation de la phospholipase glomérulaire par ces radicaux. Cet effet qui se produit vraisemblablement à la phase initiale des glomérulonéphrites expérimentales pourrait intervenir dans le mécanisme de l'inflammation.

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It is widely accepted that immunologic damage to the glomerular capillaries resulting from the deposition of circulating immune complexes depends on the local accumulation of polymorphonuclear neutrophils related to activation of the complement system [1, 2]. The toxic effect of these cells is mediated by the release of lysosomal enzymes [3] and also of oxygen radicals [4, 5]. These radicals have been identified as superoxide anion [6], hydrogen peroxide [7], and hydroxyl radical [7, 8]. Toxic oxygen metabolites released from polymorphonuclear neutrophils are highly reactive and induce cell-membrane injury as demonstrated by the increase in ^{51}Cr release from ^{51}Cr-labeled endothelial cells exposed to a oxygen-radical generating system [4]. But, this test of cytotoxicity is not specific and does not define what biochemical components of the cell are altered. Recent studies from our laboratory have shown that glomeruli isolated from rat kidney specifically bind angiotensin II [9], contain a PTH-stimulated adenylate cyclase [10], and synthesize prostaglandins (PG) from arachidonic acid [11]. Kuehl et al [12, 13] reported that hydroxyl radicals generated during the peroxidase reduction of PGG_2 to PGH_2 caused irreversible enzymic deactivation and that radical-scavenging, reducing agents stimulate the reaction by protecting this enzyme. In the present report, we demonstrate that hydrogen peroxide generated in the incubation medium of isolated glomeruli stimulates the synthesis of PG's by the glomerular cells through activation of the membrane phospholipase. This response of the glomerular cells to hydrogen peroxide is likely to occur as a consequence of polymorphonuclear neutrophil activation and could in turn control the leukocyte function because it has been shown that PGI_2 synthesized by cultured endothelial cells depressed chemotaxis and glucose oxidation [14] and that PGE_1 reduced extrusion of lysosomal enzymes [15].

Methods

Isolation of rat renal glomeruli. Rat renal glomeruli were isolated from Sprague-Dawley rats weighing 150 to 220 g according to the technique of Fong and Drummond (16) with

minor modifications. Under pentobarbital anesthesia (5 mg/100 g body weight i.p) a canula was inserted in the lower aorta. Isotonic, cold, heparinized saline (0.16 M sodium chloride, 30–50 ml) was perfused after clamping the aorta and opening the renal vein for drainage. Blanched kidneys of two rats were removed. The cortex from the four kidneys was dissected and minced to a paste-like consistency. The homogenate, suspended in 20 mM Tris-hydrochloric acid buffer, pH 7.4, containing 5 mM glucose, 135 mM sodium chloride, 10 mM potassium chloride and 10 mM sodium acetate, was pressed successively through a 106-μm sieve, which excluded the tubules, and a 50-μm sieve which retained the glomeruli. The suspension was then passed through a 25-gauge needle and centrifuged at $\times 120g$ for 90 sec. The supernatant was discarded, the pellet resuspended in the same buffer solution and passed again through the needle and centrifuged. This operation was repeated three times. Each individual preparation was checked for purity under light microscopy. Virtually no afferent and efferent arterioles could be detected. Tubular fragments were always below 2% of the total number of glomeruli.

Chemicals and radioimmunoassay reagents. PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, and TXB_2 were gifts from Dr. J. Pike (Upjohn Co, Kalamazoo, Michigan). ^{3}H-PGE_2, ^{3}H-$PGF_{2\alpha}$ (110 to 170 Ci/mmole), and 1-^{14}C-arachidonic acid (55 mCi/mmole) were purchased from the Radiochemical Centre (Amersham, United Kingdom). ^{125}I-iodinated 6-keto-$PGF_{1\alpha}$ and TXB_2 histamine conjugates were donated by Dr. F. Dray (Institut Pasteur, Paris). Anti-PGE_2, anti-$PGF_{1\alpha}$, anti-6-keto-$PGF_{1\alpha}$, and anti-TXB_2 antiserums were obtained from Institut Pasteur (Paris). These antibodies crossreact only slightly with other PG's and could be considered as specific [17–19]. The following chemicals were purchased from Sigma (St Louis, Missouri): arachidonic acid (sodium salt), xanthine oxidase (26.8 U/ml), xanthine, superoxide dismutase (2500 U/mg), and catalase (14,000 U/mg). Mepacrine was donated by Specia (Paris).

Incubation of isolated glomeruli and measurement of PG content of the incubation medium. Isolated glomeruli were incubated with or without an oxygen radical source at 37° C in room atmosphere under continuous agitation in 200 μl of 20 mM Tris-hydrochloric acid buffer (pH, 7.5) containing 5 mM glucose, 135 mM sodium chloride, 10 mM potassium chloride, 10 mM sodium acetate, and 1 mM calcium chloride. The concentration of glomerular protein could vary between different experiments (250 to 450 μg/ml) but was maintained constant (± 5%) within the same experiment. After 30 min of incubation, the preparation was centrifuged at $\times 3000g$ for 1 min and the supernatant collected.

Radioimmunoassays of PGE_2 and $PGF_{2\alpha}$ were carried out according to Dray, Charbonnel, and Maclouf [17]. Radioimmunoassays of TXB_2 and 6-keto-$PGF_{1\alpha}$ were carried out according to Sors et al [18] and Dray et al [19], respectively. The incubation medium being protein-free, these PGs were assayed in the supernatant without prior extraction at three increasing dilutions. A blank value corresponding to the incubation medium not exposed to glomeruli was prepared at each dilution used and subtracted from PG concentration when necessary. PGE_2, $PGF_{2\alpha}$, TXB_2, and 6-keto-$PGF_{1\alpha}$ productions by isolated glomeruli were studied as a function of oxygen radical concentration. All other experiments included only PGE_2 and $PGF_{2\alpha}$ assays. Glomerular protein was determined according to Lowry et al [20]. PG synthesis was expressed as nanograms per 30 min and per milligram of glomerular protein.

Source of oxygen radicals. Combination of xanthine and xanthine oxidase is a well-established oxygen-radical generating system [21, 22]. Superoxide anions are initially formed and then converted into hydrogen peroxide. There is a spontaneous conversion, which is markedly enhanced in the presence of superoxide dismutase. Hydrogen peroxide and superoxide anion radicals interact to give birth to hydroxyl radical. Hydrogen peroxide is converted into water in the presence of catalase. These two enzymes, superoxide dismutase and catalase, are widely distributed in the mammalian cells. Xanthine was used at 0.1 mM final concentration. We verified that this product was unable by itself to modify PG synthesis by isolated glomeruli. Xanthine oxidase was added at concentrations ranging between 0.84 and 26.8×10^{-4} U per tube when increasing concentrations of oxygen radicals were needed. For the other experiments, a fixed concentration of 13.4×10^{-4} U per tube was chosen. The effect of xanthine oxidase in the absence of added xanthine was measured and subtracted from that obtained with the mixture xanthine-xanthine oxidase. It represented always less than 10% of the total effect. Thus, whenever SE was given, it was calculated from the variance of this difference considered as the sum of the variance of both terms.

Uptake and release of ^{14}C-arachidonic acid by isolated glomeruli. Isolated glomeruli (approximately 3 mg/ml of glomerular protein) were incubated at room temperature in 1 ml of the same buffer as that used in PG synthesis studies in the presence of 23.5 μM ^{14}C-arachidonic acid. After 1 hour of incubation, glomeruli were washed twice with 10 ml of ice-cold buffer and then resuspended in 10 ml of buffer containing 3.5 g/liter bovine serum albumin. Aliquots (200 μl) of this suspension were incubated with or without an oxygen radical source (xanthine, 0.1 mM; xanthine oxidase, 13.4×10^{-4} U/tube) at 37° C in room atmosphere during increasing (10 to 60 min) periods of time. At the end of the incubation, each aliquot was filtered through a Millipore filter (HAWP 025) and the filter washed three times with 5 ml of 0.16 M ice-cold sodium chloride. After it had dried, ^{14}C radioactivity present on the filter was counted in 10 ml of Bray's solution [23] by standard liquid scintillation technique at 85% efficiency. Results were expressed as percentage of ^{14}C-arachidonic acid bound at the start of incubation that was released at a given time.

Results

PG production by isolated glomeruli in the presence of an oxygen radical generating system. The synthesis rate of the four PG's measured, PGE_2, $PGF_{2\alpha}$, TXB_2, and 6-keto-$PGF_{1\alpha}$, was progressively stimulated in the presence of a fixed dose of xanthine and increasing concentrations of xanthine oxidase, this corresponding to increasing rates of formation of oxygen radicals. At a concentration of xanthine oxidase of 26.8×10^{-4} U/tube, the degree of stimulation was similar for all these PG's ranging between 1.74 and 1.98 (Fig. 1). Because productions of PGE_2 and $PGF_{2\alpha}$ were the greatest in absolute value, only one or both of these PG's were measured in the following experiments. PGE_2 accumulated progressively with time in the incubation medium of isolated glomeruli. When the mixture xanthine-xanthine oxidase was added, PGE_2 production was clearly enhanced (1.5 times the control value at 30 min). The degree

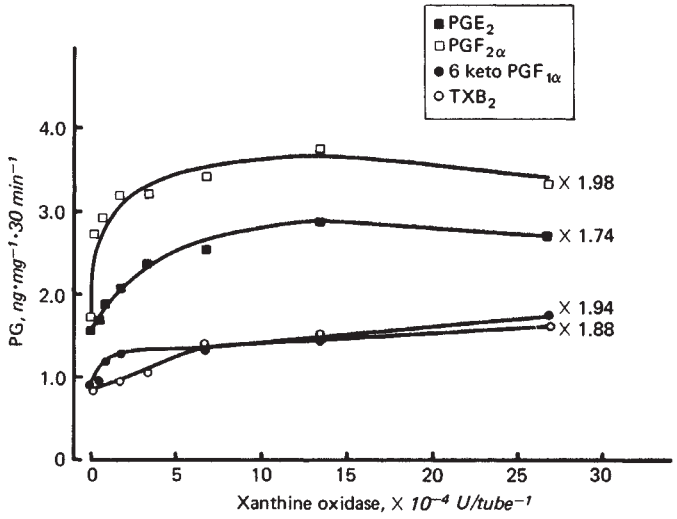

Fig. 1. *PGE_2, $PGF_{2\alpha}$, 6 keto $PGF_{1\alpha}$, and TXB_2 production by isolated glomeruli after 30 min of incubation as a function of increasing doses of xanthine oxidase in the presence of 0.1 mM xanthine (system generating increasing concentrations of oxygen radicals).* Each value is the mean of triplicates. The maximum degrees of stimulation for each PG are indicated. The correlation coefficients between the synthetic rate of each PG tested and the concentration of xanthine oxidase are all highly significant ($P < 0.01$).

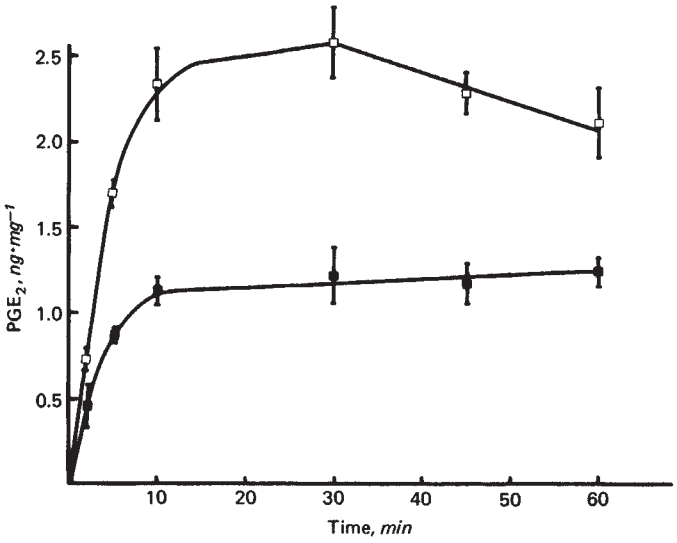

Fig. 2. *Time-course of PGE_2 accumulation in the incubation medium of isolated glomeruli with* (open squares) *and without* (closed squares) *an oxygen radical generating system (0.1 mM xanthine plus 13.4 × 10^{-4} U of xanthine oxidase per tube).* Each point is the mean of triplicates and each vertical bar twice the SE. Two-factor (time, oxygen radicals) analysis of variance with replications showed that the effect of oxygen radicals was highly significant ($P < 0.01$).

of stimulation slightly decreased after 30 min of incubation (Fig. 2). Basal PGE_2 synthesis, related to 1 mg of glomerular protein, ranged between 0.9 and 0.6 ng per 30 min when the concentration of glomerular protein increased from 0.2 to 1.2 mg/ml. After addition of the oxygen-radical generating system, a marked stimulation of PGE_2 synthesis was observed at the lowest concentrations of glomerular protein. There was an inverse relationship between the degree of stimulation and the concentration of glomerular protein (Fig. 3). This was interpreted as being due to the increasing amount of glomerular enzymes (superoxide dismutase and catalase) destroying the oxygen radicals that were generated at a constant rate. Because of this high dependency of the effect of oxygen radicals on the concentration of glomerular protein, we were very careful to maintain constant this concentration within a given experiment.

To appreciate which oxygen byproduct was active on PG synthesis, we studied the effects of the two main cell enzymes playing a role in the metabolism of these radicals, superoxide dismutase and catalase. Increasing concentrations of catalase progressively inhibited the stimulation of PGE_2 (Fig. 4) and $PGF_{2\alpha}$ (Fig. 5) synthesis in the presence of the oxygen radical generating system. At the highest concentration of this enzyme tested, the effect of these radicals was abolished. In the absence of oxygen byproducts, the effect of catalase on PGE_2 and $PGF_{2\alpha}$ synthesis was slight or nil, respectively. The effects of superoxide dismutase were more complex. For the lowest concentrations of this enzyme, there was a moderate stimulation of the synthesis of both PG's followed at higher concentrations by a return to the control values. This effect was clear only in the presence of oxygen radicals (Figs. 4 and 5). These experiments demonstrate that superoxide anion was not responsible for the stimulation of PG synthesis because this stimulation persisted and even was stimulated in the presence of superoxide dismutase. Hydrogen peroxide and the products

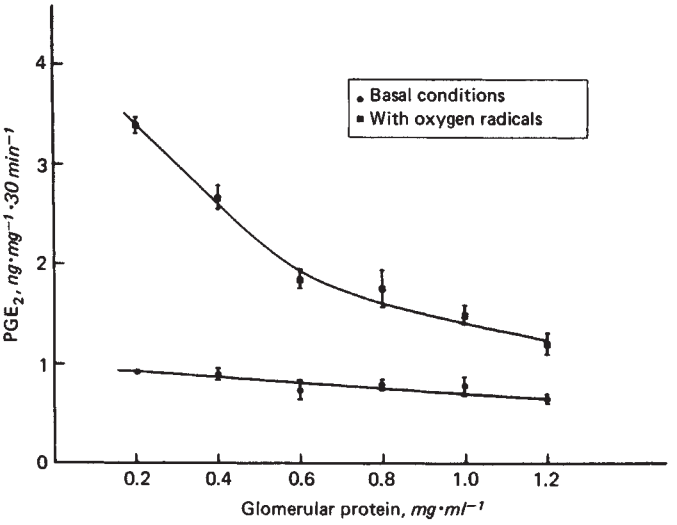

Fig. 3. *PGE_2 production by isolated glomeruli after 30 min of incubation as a function of increasing concentrations of glomerular protein with and without an oxygen radical generating system (0.1 mM xanthine plus 13.4 × 10^{-4} U of xanthine oxidase per tube).* Each point is the mean of triplicates and each vertical bar twice the SE. The correlation coefficient between PGE_2 synthetic rate and glomerular protein concentration in the presence of oxygen radicals is highly significant ($P < 0.01$).

of its interaction with superoxide anion, hydroxyl radical, and singlet oxygen should thus play a role. To find which of them was active, we studied the effects of two hydroxyl radical scavengers, chlorpromazine [24] and mannitol [25], after verification that neither modifies PG synthesis in control conditions. Neither mannitol at two concentrations nor chlorpromazine had any influence on the degree of stimulation of PGE_2 synthesis in

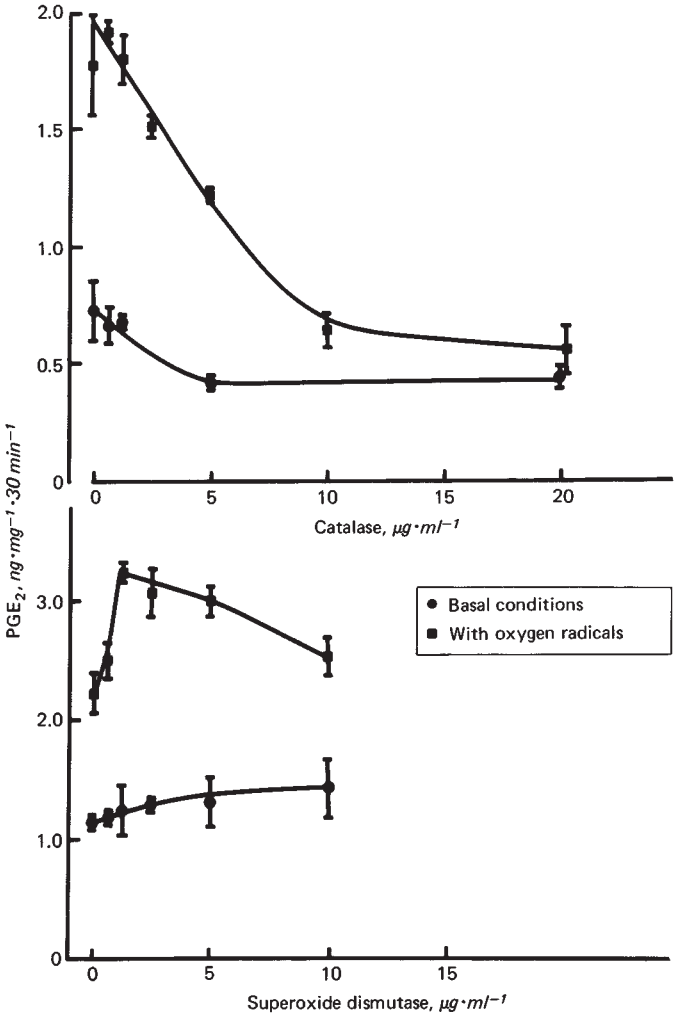

Fig. 4. *PGE_2 production by isolated glomeruli under basal conditions and in the presence of an oxygen radical generating system (0.1 mM xanthine plus 13.4 × 10^{-4} U of xanthine oxidase per tube) as a function of increasing doses of either catalase* (upper part) *or superoxide dismutase* (lower part). Each point is the mean of triplicates and each vertical bar twice the SE. The correlation coefficient between PGE_2 synthetic rate and catalase concentration in the presence of oxygen radicals is highly significant ($P < 0.01$).

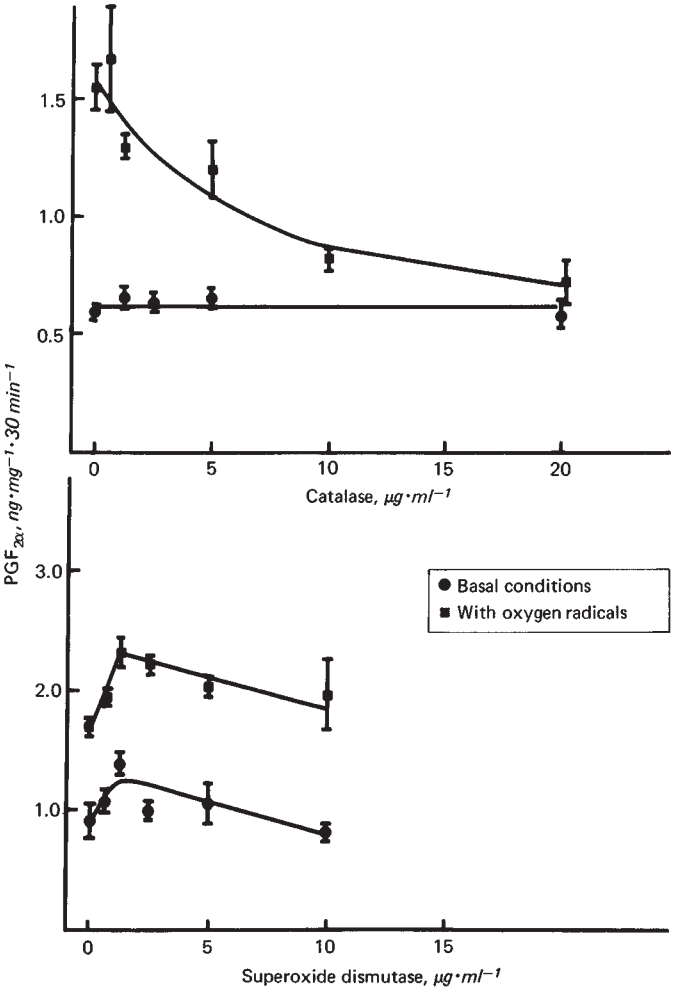

Fig. 5. *$PGF_{2\alpha}$ production by isolated glomeruli under basal conditions and in the presence of an oxygen radical generating system (0.1 mM xanthine plus 13.4 × 10^{-4} U of xanthine oxidase per tube as a function of increasing doses of either catalase* (upper part) *or superoxide dismutase* (lower part). Each point is the mean of triplicates and each vertical bar twice the SE. The correlation coefficient between $PGF_{2\alpha}$ synthetic rate and catalase concentration in the presence of oxygen radicals is highly significant ($P < 0.01$).

the presence of the oxygen radical generating system (Table 1). These experiments must be interpreted cautiously, for these drugs cannot be considered as specifically trapping hydroxyl radicals. This suggests, however, that hydrogen peroxide or singlet oxygen or both were the responsible agents. The role of hydrogen peroxide was confirmed by testing the effects of increasing concentrations of this oxygen byproduct directly added to the incubation medium. Hydrogen peroxide stimulated both PGE_2 and $PGF_{2\alpha}$ synthesis at concentrations below 100 μM. A decrease in the degree of stimulation occurred at higher concentrations (Fig. 6).

The following experiments were designed to determine the site of action of hydrogen peroxide on PG synthesis. PGE_2 production was studied at increasing concentrations of its precursor arachidonic acid in the presence of two concentrations of oxygen radicals or of 50 μM hydrogen peroxide. Stimulation of PGE_2 production expressed as percentage of the control value (without the mixture xanthine-xanthine oxidase or hydrogen peroxide) diminished rapidly when arachidonic acid was added (Fig. 7). Arachidonic acid at 5 μg/ml provoked no stimulation. This suggested an effect of hydrogen peroxide at an early stage of PG synthesis prior to arachidonic acid cyclooxygenase and probably on arachidonic acid release. Because this release has been shown to be influenced by calcium ions [26], we studied the effects of verapamil (5 μM), an inhibitor of calcium transport, on the stimulation of PGE_2 and $PGF_{2\alpha}$ synthesis by oxygen radicals. Verapamil did not reduce the stimulation of PG synthesis by oxygen radicals when it was added in basal conditions (without arachidonic acid). No stimulation of PG synthesis by oxygen radicals was observed in the presence of arachidonic acid with and without verapamil (Table 2). We also studied the effect of mepacrine, an inhibitor of

Table 1. Effects of OH trappers on PGE_2 synthesis by isolated glomeruli in the presence of an oxygen radical generating system[a]

Drugs tested	PGE_2 synthesis		
	Control	With oxygen radicals	Percent of control production
	$pg \cdot 30\ min^{-1} \cdot mg^{-1}$		
Mannitol (10 mM)			
with	750	1904	254
without	685	1685	246
Mannitol (100 μM)			
with	894	2749	307
without	842	2679	318
Chlorpromazine (0.25 mM)			
with	1294	3441	266
without	1024	3000	293

[a] Means from duplicates are given. Results were analyzed according to a three-factor analysis of variance with replication. There was no ignificant interaction between the factor "absence or presence of drug" and the factor "free oxygen radicals."

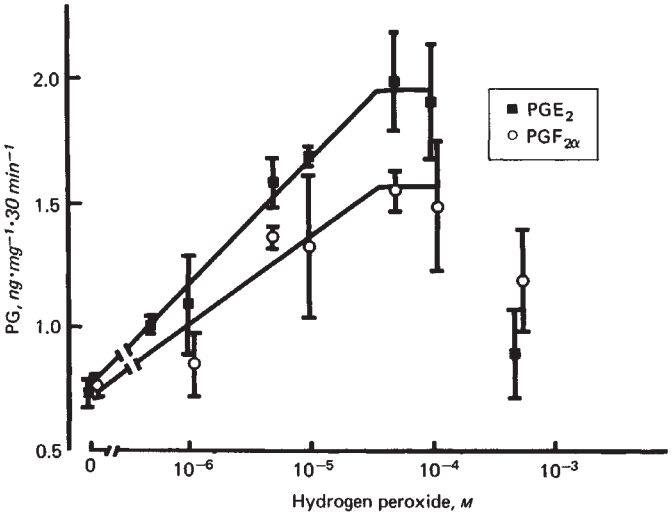

Fig. 6. *PGE_2 and $PGF_{2\alpha}$ productions by isolated glomeruli after 30 min of incubation as a function of increasing concentrations of hydrogen peroxide.* Each point is the mean of triplicates and each vertical bar twice the SE. The correlation coefficients between either PGE_2 or $PGF_{2\alpha}$ synthetic rates and hydrogen peroxide concentrations (0 to 100 μM) are highly significant ($P < 0.01$).

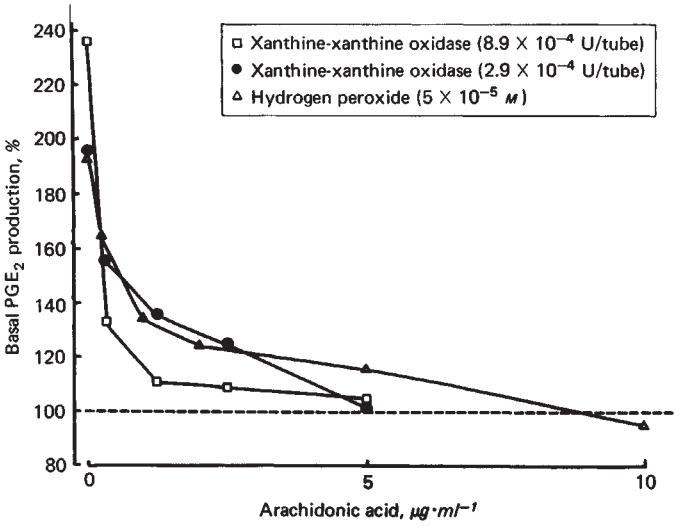

Fig. 7. *Stimulation of glomerular PGE_2 production after 30 min of incubation by two concentrations of oxygen radicals (0.1 mM xanthine plus 2.9 × 10^{-4} or 8.9 × 10^{-4} U of xanthine oxidase per tube) or hydrogen peroxide (5 × 10^{-5} M) in the presence of increasing concentrations of arachidonic acid.* Each point is the mean of duplicates. The correlation coefficients between the percentage of stimulation of basal PGE_2 synthetic rate in each condition and arachidonic acid concentrations are highly significant ($P < 0.01$).

phospholipase activity (Fig. 8). PGE_2 production decreased linearly in the presence of increasing doses of mepacrine both with and without 50 μM hydrogen peroxide. But the slope of the regression line was clearly greater ($P < 0.01$) with hydrogen peroxide (− 1045 ng of PGE_2 produced per 10^{-5} M mepacrine) than under basal conditions (− 387 ng of PGE_2 produced per 10^{-5} M mepacrine).

Release of ^{14}C-arachidonic acid from isolated glomeruli in the presence of an oxygen radical generating system. The effect of a mixture of two fixed doses of xanthine and xanthine oxidase on the release of ^{14}C-arachidonic acid is shown in Fig. 9. ^{14}C-Arachidonic acid progressively accumulated in the incubation medium with and without this source of oxygen radicals. The rate of release was rapid during the first 15 min and then decreased. A plateau was reached at 50 min. Generation of oxygen radicals produced an increase of the release. The difference between the percentage of incorporated ^{14}C-arachidonic acid released under the two conditions studied was nearly constant, because both curves were parallel over the period 10 to 60 min. This supplementary amount of ^{14}C-arachidonic acid released represented approximately 20% of the total radiolabeled fatty acid incorporated in the glomeruli.

Discussion

The effects of oxygen radicals on PG synthesis have previously been studied under limited conditions. Only oxygen byproducts generated during physiologic metabolic conditions within the cells have been considered, and their role has been indirectly estimated from the effects of hydroxyl radical scavengers or enzymes such as catalase and superoxide dismutase destroying specifically one of these oxygen byproducts. These studies have shown that hydroxyl radicals, formed during peroxidase-stimulated conversion of PGG_2 in PGH_2 and proba-

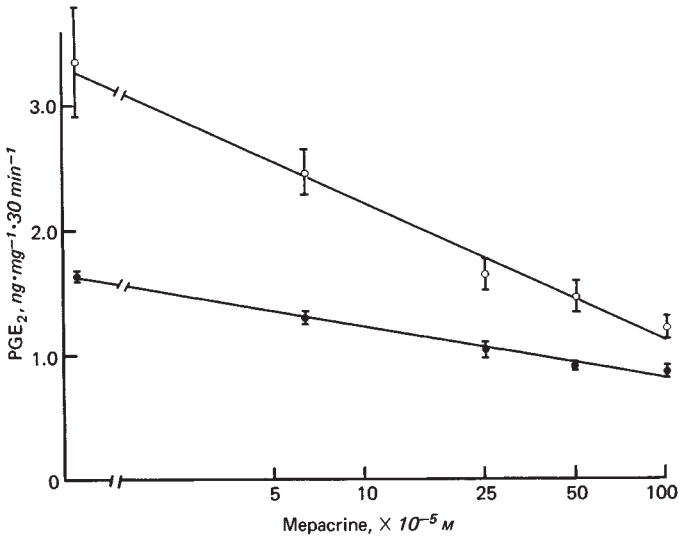

Fig. 8. *PGE_2 production by isolated glomeruli under basal conditions* (closed circles) *and in the presence of hydrogen peroxide (5×10^{-5} M)* (open circles) *as a function of increasing doses of mepacrine.* Each point is the mean of triplicates and each vertical bar twice the SE. The correlation coefficients between PGE_2 synthetic rate and mepacrine concentration are highly significant ($P < 0.01$).

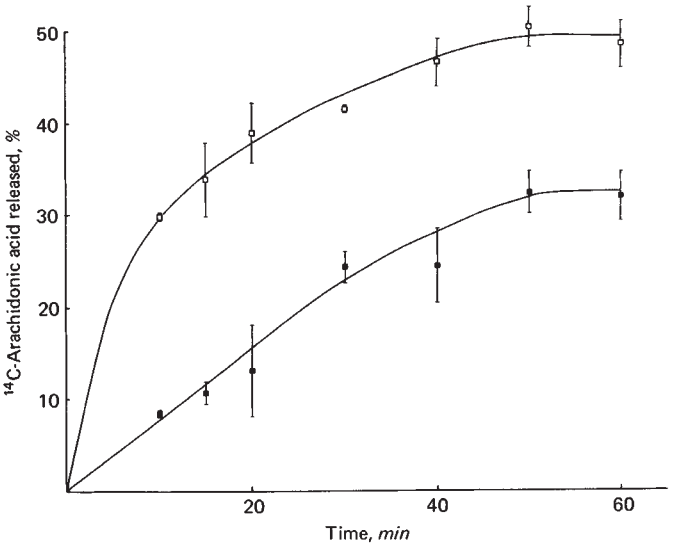

Fig. 9. *Percentage of incorporated ^{14}C-arachidonic acid released from isolated glomeruli with time with* (open squares) *and without* (closed squares) *an oxygen-radical generating system (0.1 mM xanthine plus 13.4×10^{-4} U of xanthine oxidase per tube.* Each point is the mean of triplicates and each vertical bar twice the SE. Two-factor (time, oxygen radicals) analysis of variance with replication showed that the effect of oxygen radicals is highly significant ($P < 0.01$).

bly hydroperoxyeicosatetraenoic acid (HPETE) into hydroxyeicosatetraenoic acid (HETE) produced an irreversible deactivation of both peroxidase and cyclooxygenase and thus an inhibition of PG synthesis [12, 13]. Other studies [27] performed with the same material, microsomes from seminal vesicles, demonstrated that the effects of hydroxyl radical scavengers were different according to the dose used. Biosynthesis was stimulated only at low concentrations, whereas it was inhibited at high concentrations, thus suggesting that, at least under some conditions, an oxidizing agent was necessary to obtain a full stimulation of cyclooxygenase. In this respect, the role of hydrogen peroxide was proposed by Vargaftig, Tranier, and Chignard [28] as a mediating agent for platelet aggregation by arachidonic acid. The purpose of our experiments was quite different, for we wished to study the effects of oxygen radicals generated outside the cells on PG synthesis by isolated glomeruli. This preparation consists of intact cells including all the components of the PG synthesis pathway. This experimental protocol thus mimics more closely the conditions observed during polymorphonuclear leukocyte accumulation in the glomerular capillaries. Our results are also different from those previously reported. We have shown that hydrogen peroxide stimulates phospholipase activity and thus enhances PG synthesis through an increase in the availability of arachidonic acid. We do not know to what extent the oxygen radicals generated outside the cells act on the microsomal cyclooxygenase and peroxidase enzymes. It is logical to estimate that they modify primarily phospholipase activity, which is present in the cell membrane. But, the shape of the curves obtained in the presence of increasing concentrations of hydrogen peroxide (Fig. 6) suggests that the lesser degree of stimulation of PGE_2 and $PGF_{2\alpha}$ synthesis observed at the highest concentrations of this oxygen byproduct may be due to an increase in its intracellular concentration acting on cyclooxygenase activity.

We have also demonstrated that the main oxygen byproduct responsible for the increase in PG synthesis observed in our studies was hydrogen peroxide. This conclusion relies on the following: *(1)* catalase suppressed the effects of the oxygen radical generating source, whereas superoxide dismutase produced a slight increase; *(2)* hydroxyl radical scavengers were inactive; *(3)* hydrogen peroxide itself stimulated PGE_2 and $PGF_{2\alpha}$ synthesis. These arguments do not rule out a role for singlet oxygen, which has been shown as stimulating PG synthesis by sheep vesicular gland microsomes [29], but contrary to hydrogen peroxide a direct demonstration of its effect was not possible. Hydrogen peroxide was active between 1 and 100 μM. This range of concentration appears to be close to that which can be generated in the glomerular capillaries by the leukocytes. Nathan et al [30] estimated that 6.6×10^5 polymorphonuclear leukocytes generated 2.3×10^{-5} moles of hydrogen peroxide over 1 hour. In our system (5×10^{-5} M hydrogen peroxide), the amount of hydrogen peroxide present in 1 ml would need about 3000 leukocytes. Because the concentration used for isolated glomeruli is 300 μg/ml, corresponding roughly to 3000 glomeruli, this corresponds to one leukocyte per glomerulus. This figure agrees with the physiologic conditions: 7000 leukocytes/mm^3 and 200×10^3 μm^3 for the glomerular capillary volume of the rat [31]. This calculation does not take into account the catabolism of hydrogen peroxide by the glomerular catalase, but, on the other hand, the number of leukocytes present in the glomerular capillaries markedly increases at the early stage of glomerulonephritis. We can therefore estimate that the concentrations of hydrogen peroxide used in this study are consistent with those which may be expected in vivo. Hydrogen peroxide seems to be quite unreactive and in particular does not induce lipid peroxidation [32]. Toxicity has been attributed to its metabolites such as hydroxyl radicals and singlet oxygen, which are generated in vivo [33]. The effect of

Table 2. Effects of verapamil (5 μg/ml) on the stimulation of PGE_2 and $PGF_{2\alpha}$ synthesis by isolated glomeruli in the presence of an oxygen radical generating system[a]

	PGE_2 synthesis			$PGF_{2\alpha}$ synthesis		
	Control	With oxygen radicals	Percent of control production	Control	With oxygen radicals	Percent of control production
	$pg \cdot 30\ min^{-1} \cdot mg^{-1}$			$pg \cdot 30\ min^{-1} \cdot mg^{-1}$		
Basal conditions						
without verapamil	503 ± 118	968 ± 32	193	636 ± 66	1482 ± 206	233
with verapamil	321 ± 20	609 ± 8	190	628 ± 164	1548 ± 114	247
With arachidonic acid (5 μg/ml)						
without verapamil	6419 ± 335	5817 ± 296	91	2512 ± 44	2605 ± 182	104
with verapamil	5085 ± 387	4310 ± 295	85	3372 ± 165	3411 ± 248	101

[a] Means ± SE from three values are given. Results were analyzed separately for PGE_2 and $PGF_{2\alpha}$ according to a three-factor analysis of variance with replication. There was no significant interaction between the factor "verapamil" and the factor "oxygen radicals."

hydrogen peroxide on PG production as a consequence of the stimulation of phospholipase activity is thus unexpected. But as suggested by our quantitative estimation of hydrogen peroxide concentrations, this effect could play a role in renal diseases and possibly in physiologic conditions. Recently, Polgar and Taylor [34] have shown that the stimulation of PG synthesis by ascorbic acid in human lung fibroblasts was in fact due to the formation of hydrogen peroxide.

Hydrogen peroxide appears to stimulate phospholipase activity in glomeruli. Mepacrine, an inhibitor of phospholipase activity, was clearly more active in the presence of hydrogen peroxide. This is in favor of an action of this oxygen byproduct at the stage of phospholipase. Furthermore, the effect of hydrogen peroxide or of oxygen radical generating source was apparent only when exogenous arachidonic acid was not added. This suggests that the mechanism of action of hydrogen peroxide is to increase the availability of arachidonic acid. Oxygen radicals also stimulated the release from isolated glomeruli of previously incorporated ^{14}C-arachidonic acid. In this condition, ^{14}C appears in the incubation medium either in the form of arachidonic acid or its metabolites when phospholipase is activated. Thus, the amount of ^{14}C appearing in the incubation medium is a measure of the extent of phospholipase activation. The percentage of ^{14}C released in basal conditions represented about 30% of the total ^{14}C incorporated. This may appear as considerable; but ^{14}C-arachidonic acid released was continuously trapped by albumin present in the incubation medium, which maintained free arachidonic acid at a low concentration and prevented any reincorporation. Phospholipase activity is regulated by calcium [26] and also by several hormonal peptides [35]. Because verapamil did not modify the stimulatory effect of oxygen free radicals (Table 2), it is likely that hydrogen peroxide does not act via a change in calcium transport across the membranes of the glomerular cells. In the in vitro conditions used, the mechanism of action of hydrogen peroxide was clearly independent of the polypeptide hormones.

The possible consequences of the hydrogen-peroxide-dependent increase in PG synthesis by isolated glomeruli are, first of all, those involving glomerular function. PGE_2 and PGI_2 have been shown to stimulate glomerular adenylate cyclase [36]. Other effects of glomerular PG's, either directly or through cyclic AMP production, could be a decrease in the glomerular ultrafiltration coefficient [37] and stimulation of renin production by the juxtaglomerular apparatus [38]. Several lines of evidence exist implicating PG's as mediators in inflammation. At least at the early stage of the inflammatory process, PG's cause vasodilatation and increase capillary permeability [39]. These events could thus be the consequence of the local accumulation of polymorphonuclear neutrophils via the production of hydrogen peroxide. The role of PG's in the development of the glomerular lesions of human glomerulonephritis is also supported by the beneficial effect of nonsteroid antiinflammatory agents on the progression of renal function impairment [40]. PG's synthesized by the glomeruli may, in turn, regulate further leukocyte activation. The release of the proinflammatory lymphokines [41] and of lysosomal enzymes [15] is inhibited by PG's of the E series. PGI_2 also inhibits polymorphonuclear leukocyte adherence [42] and chemotaxis [14]. This suggests an homeostatic feedback mechanism in which complement-activated polymorphonuclear leukocytes produce hydrogen peroxide, which stimulates the synthesis by glomeruli of PG's, which, in turn, depress leukocyte activation.

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